AD-A171 213 POTENTIAL OF MICROBORE HPLC (HIGH PERFORMANCE LIQUID CHROMATOGRAPHY) FOR (U) ROSENSTIEL SCHOOL OF MARINE AND ATMOSPHERIC SCIENCE MIAMI FL K MOPPER 04 AUG 86 1/1

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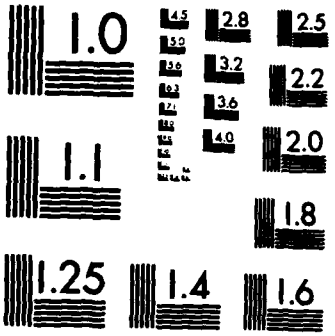

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FINAL TECHNICAL REPORT
for Contract #N00014-84-K-0733
Office of Naval Research

Project Title: Potential of Microbore HPLC for Trace Organic Analysis in the Ocean

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Long-Range Objectives

The adaptation of modern chromatographic techniques, especially capillary gas chromatography and high performance liquid chromatography (HPLC), to seawater analysis has lead to new insights into chemical and biological oceanographic processes. In this project the main long-range objective is to extend the role of HPLC by exploring the applicability of microbore HPLC to trace organic analysis of seawater. This technique offers substantially greater sensitivity and separation efficiency than conventional HPLC and, furthermore, the entire effluent of microbore columns may be fed into selective detectors, such as mass spectrometers and flame-base detectors. Initially, the microbore HPLC system will be used for the analysis of carboxylic acids as part of our ONR-funded project. Other potential marine applications, such as the analysis of amino acids, pigments, photosensitizers, thiols, macromolecules and metal-organic complexes, will also be investigated as opportunities arise. In order to obtain information on the nature of the substances eluting off the microbore column the effluent will be analyzed spectroscopically with an on-the-fly UV-VIS diode array detector. Finally, we will explore (cooperatively with Zika's group) potential marine applications of laser excitation fluorescence detection in conjunction with microbore HPLC.

Short-Term Objectives

The main stort-term objective is to carefully evaluate commercially available HPLC instrumentation and diode array detectors in terms of their suitability for microbore work.

Summary of Work to Date

Since low flow rates (1-100 µl . min^{-1}) are used in microbore HPLC, it is an instrumentally demanding technique. Conventional HPLC equipment cannot be

used without substantial modifications. Fortunately, advances in instrumentation such as detectors with low microliter volume flow cells, reliable and pulse-free pumps operating in the microliter per min range, small volume injection valves, and improved column packing techniques have recently made it possible to explore the potential uses of microbore HPLC without substantial investment of time in instrumentation modification and design.

Although several commercial microbore HPLC systems currently available meet the demanding requirements of the technique, they are limited in one important aspect. With a few exceptions, these systems operate isocratically (i.e., a single mobile phase). This is equivalent to a GC system which can only operate isothermally. In HPLC, gradient elution via mobile phase programming is extremely important for the analysis of complex mixtures (e.g. seawater samples) since the technique allows control over selectivity, increases peak capacities and reduces overall analysis times. Gradient elution in microbore HPLC is feasible and a number of manufacturers claim to have instruments that have this capability.

It was initially estimated that 9-12 months would be needed to evaluate commercially available instruments prior to the actual acquisition. However, due to scheduling problems with various vendors for the demonstration of different pieces of equipment as well as the unexpectedly poor performance (in terms of microbore work) of most of the equipment that was demonstrated, an additional 6 months was required for testing.

In total, nine instruments were evaluated. The instruments were designed to generate mobile phase gradients based on one of three ways:

1) single pump system with mobile phase programming on the low pressure side using computer controlled solenoid valves; the generated solvent gradient then passes through the pump;

2) single pump system in which a loop reservoir is used to store the generated mobile phase gradient on the low pressure side of the pump (with solenoid valves); by use of a programmable 10-port valve, the loop is then switched over to the high pressure side of the pump; thus the generate gradient is not altered by passing through the pump;

3) two pump system in which the mobile phase gradient is generated by computer programming the pump speeds, followed by low dead volume, high pressure mixing.

The latter method (two pump system) gave by far the best results in terms of low system dead volume, and control over the gradient composition, even at low flow rates (e.g., 5μl . min^{-1}). A comparison between the programmed mobile phase gradient and the actual gradient generated (prior to the column) at a total flow rate of 10 μl . min^{-1} is shown in Fig. 1. Because of these excellent results, we decided to proceed with this alternative. The appropriate chromatographic equipment has been ordered from Gilson International and will be shipped 8/86. The UV-VIS diode array detection system with microbore flow cell was ordered from Hewlett-Packard. The latter equipment has arrived and we are currently setting it up.

The short-term objectives of this project have been met. The long-range objectives will be satisfied as part of our on-going ONR funded research on the cycling of carboxylic acids in the sea and as part of the SOLARS Program. It is anticipated that microbore HPLC will be of considerable benefit to this work and that the first studies employing this methodology will be submitted for publication by the end of calender year 1986 or beginning of 1987.

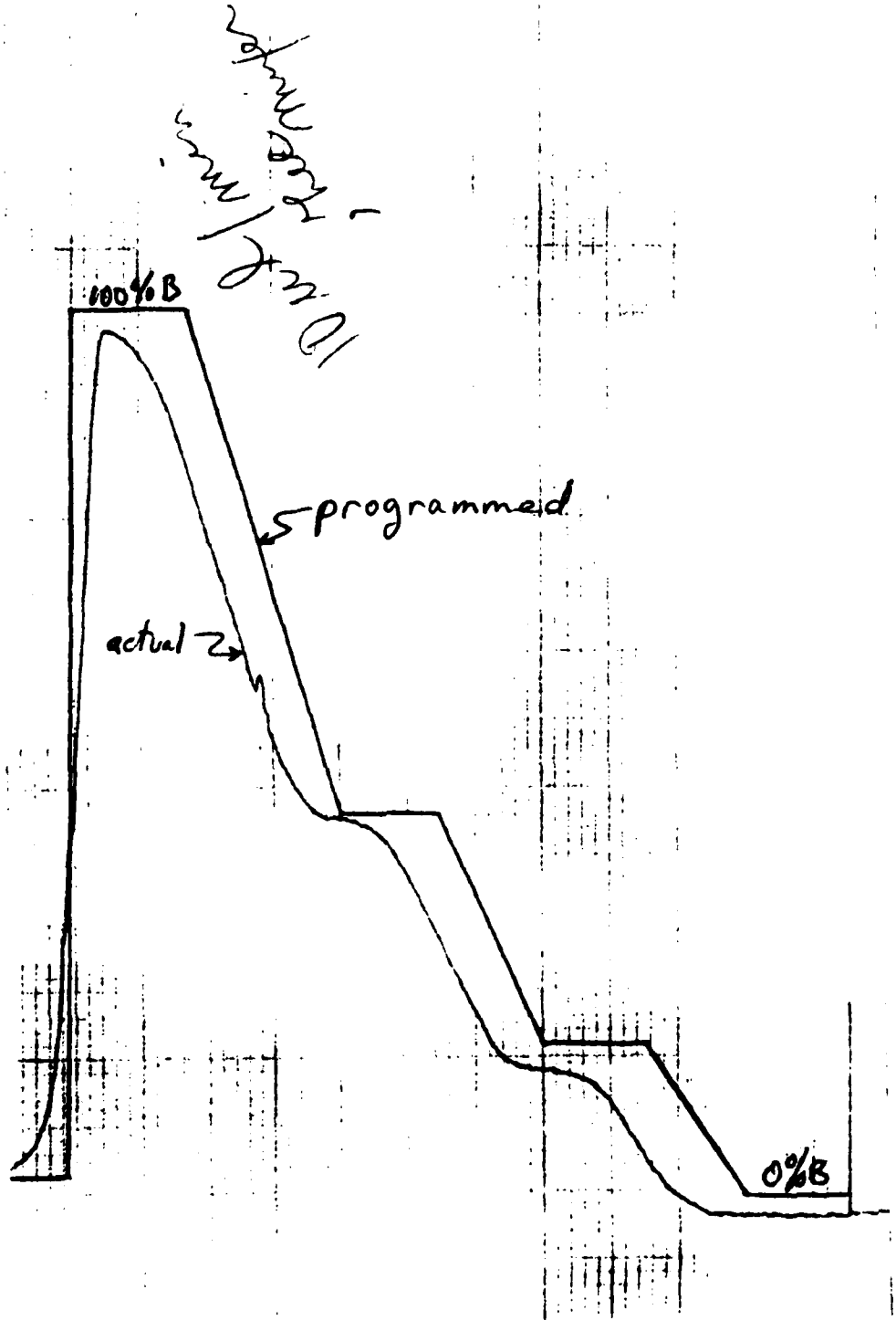

Fig. 1 Programmed mobile phase gradient and actual gradient generated at 10 $\mu l \cdot min^{-1}$ using a two pump microbore HPLC system (Gilson) and a low dead volume static mixer (Lee).

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